

**WHAT IS CLAIMED IS:**

1. A hydrolytically stable isoelectric hydrogel material having a pI value in the range of  $11.5 < \text{pI} < 14$ , wherein the hydrolytically stable isoelectric hydrogel material comprises:

(a) an isoelectric compound having a pI value in the range of  $11.5 < \text{pI} < 14$ , wherein the pI is established by a substantially permanently cationic group and two hydroxyl groups with  $\text{pK}_a$  values in the range of  $11.5 < \text{pK}_a < 14$ ,

(b) the isoelectric compound having an additional reactive group, and  
(c) a cross-linker substantially free of ionic functional groups and comprising two groups able to react with the isoelectric compound without altering the pI value of the isoelectric compound by more than about 1 pH unit,

wherein the pI value of the isoelectric hydrogel is conferred by the pI value of the isoelectric compound.

2. The hydrolytically stable isoelectric hydrogel material of claim 1, further comprising:

(d) a hydrolytically stable hydrophilic polymer, the polymer comprising two or more reactive groups and being substantially free of ionic functional groups, wherein the cross-linker is substantially free of ionic functional groups and comprising two groups able to react with both the isoelectric compound and the hydrolytically stable polymer without altering the pI value of the isoelectric compound by more than about 1 pH unit.

3. The isoelectric hydrogel material of claim 1, wherein the pI value of the isoelectric hydrogel is changed by changing identity of the isoelectric compound.

4. The isoelectric hydrogel material of claim 1, wherein the isoelectric compound is present at a concentration equal to or higher than necessary to create an aqueous solution of the isoelectric compound with a pH equal to the pI value of the isoelectric compound.

5: The hydrolytically stable isoelectric hydrogel material of claim 2,

wherein:

the isoelectric compound is a native or derivatized carbohydrate or polyhydroxy compound;

the permanently cationic functional group is a quaternary ammonium group;

the additional reactive group of the isoelectric compound is a hydroxyl group,

the hydrolytically stable hydrophilic polymer is an oligosaccharide or a synthetic polymer containing multiple hydroxyl groups, and

the cross-linker has two or more, reactive groups selected from the group consisting of an aldehyde, epoxy, halo, alkylsulfonyl, and arylsulfonyl groups.

6. The hydrolytically stable isoelectric hydrogel material of claim 5,

wherein:

the native or derivatized carbohydrate or polyhydroxy compound is selected from the group consisting of native or derivatized monosaccharides, native or derivatized disaccharides, native or derivatized trisaccharides, native or derivatized oligosaccharides, native or derivatized polysaccharides, native or derivatized cyclodextrins, native or derivatized maltodextrins, native or derivatized amyloses, native or derivatized dextrans, native or derivatized starches, native or derivatized celluloses, and native or derivatized guar gums,

the quaternary ammonium group comprises one or more substituents selected from the group consisting of methyl, ethyl, propyl, butyl, higher alkyl, hydroxymethyl, hydroxyethyl, hydroxypropyl, hydroxybutyl, higher hydroxyalkyl, saturated ring systems, and unsaturated ring systems,

the hydrolytically stable hydrophilic polymer is selected from the group consisting of hydroxymethyl cellulose, hydroxyethyl cellulose, and poly(vinyl alcohol), and

the cross-linker is selected from the group consisting of glutaraldehyde, glycerol-1,3-diglycidyl ether, bis-(2-bromoethylene)-ethyleneglycol, and bis(2-tosylethylene)-ethyleneglycol.

**7. The hydrolytically stable isoelectric hydrogel material of claim 6,**

wherein:

the isoelectric material is a glycidyl trimethylammonium derivative of beta-cyclodextrin,

the hydrolytically stable hydrophilic polymer is poly(vinyl alcohol),  
and

the cross-linking agent is glycerol-1,3-diglycidyl ether.

**8. A hydrolytically stable isoelectric membrane having a pI value in the range of  $11.5 < \text{pI} < 14$  comprising:**

the hydrolytically stable isoelectric hydrogel of claim 1, supported on a hydrolytically stable, porous, inert or reactive substrate.

**9. The hydrolytically stable isoelectric hydrogel membrane of claim 8,**  
wherein the porous substrate is selected from the group consisting of poly(vinyl alcohol), partially or fully hydrolyzed poly(epihalohydrin), partially or fully hydrolyzed poly(epihalohydrin-co-ethylene oxide), poly(vinyl sulfone), and poly(ether-ether ketone).

**10. A hydrolytically stable hydrophilic isoelectric hydrogel material having a pI value in the range of  $11.5 < \text{pI} < 14$  comprised of:**

(a) a precursor comprising a hydroxyl group with a pKa value in the range of  $11.5 < \text{pKa} < 14$  and an additional reactive group,

(b) a derivatizing agent comprising a permanently cationic functional group and an additional reactive group, and

(c) a cross-linker substantially free of ionic functional groups and comprising two groups able to react with the isoelectric compound without altering the pI value of the isoelectric compound by more than about 1 pH unit,

wherein the derivatizing agent is structurally different from the precursor,

wherein the pI value of the isoelectric hydrogel is conferred by the concentration of the derivatizing agent and the precursor and the pKa value of the hydroxyl group of the precursor, and

wherein the cross-linker is structurally different from both the precursor and the derivatizing agent.

**11.** The hydrolytically stable hydrophilic isoelectric hydrogel material of claim 10, further comprising:

(d) a hydrolytically stable hydrophilic polymer having two or more reactive groups that is substantially free of ionic functional groups, wherein the hydrophilic polymer is structurally different from the precursor and the derivatizing agent;

wherein the cross-linker is substantially free of ionic functional groups and having two groups able to react with both the precursor and the hydrolytically stable hydrophilic polymer.

**12.** The hydrolytically stable hydrophilic isoelectric hydrogel material of claim 11, wherein:

the precursor is a native or derivatized carbohydrate or polyhydroxy compound,

the derivatizing agent comprises a quaternary ammonium group,

the reactive group of the derivatizing agent is selected from the group consisting of hydroxyl, aldehyde, epoxy, halo, alkylsulfonyl, and arylsulfonyl groups,

the hydrolytically stable hydrophilic polymer is an oligosaccharide or a synthetic polymer containing multiple hydroxyl groups, and

the cross-linker has two or more reactive groups selected from the group consisting of aldehyde, epoxy, halo, alkylsulfonyl, and arylsulfonyl groups.

**13.** The hydrolytically stable hydrophilic isoelectric hydrogel material of claim 12, wherein:

the precursor is selected from the group consisting of native or derivatized monosaccharides, native or derivatized disaccharides, native or derivatized trisaccharides, native or derivatized oligosaccharides, native or derivatized polysaccharides, native or derivatized cyclodextrins, native or derivatized maltodextrins, native or derivatized amyloses, native or derivatized dextrins, native or derivatized starches, native or derivatized celluloses, and native or derivatized guar gums,

the quaternary ammonium group of the derivatizing agent contains one or more substituents selected from the group consisting of methyl, ethyl, propyl, butyl, higher alkyl, hydroxymethyl, hydroxyethyl, hydroxypropyl, hydroxybutyl, higher hydroxyalkyl groups, saturated ring systems, and unsaturated ring systems,

the cross-linker is selected from the group consisting of glutaraldehyde, glycerol-1,3-diglycidyl ether, bis-(2-bromoethylene)-ethyleneglycol, and bis(2-tosylethylene)-ethyleneglycol, and

the hydrolytically stable polymer is selected from the group consisting of hydroxyethyl cellulose, hydroxymethyl cellulose, and poly(vinyl alcohol).

**14.** The hydrolytically stable hydrophilic isoelectric hydrogel material of claim 13, wherein:

the precursor is beta-cyclodextrin,

the derivatizing agent is glycidyl-trimethylammonium chloride,

the cross-linker is glycerol-1,3-diglycidyl ether, and

the hydrolytically stable hydrophilic polymer is poly(vinyl alcohol).

**15.** The hydrolytically stable hydrophilic isoelectric hydrogel material of claim 13, wherein:

the precursor is poly(vinylalcohol), and

the cross-linker is glycerol-1,3-diglycidyl ether and the derivatizing agent is glycidyl-trimethylammonium chloride.

**16.** A hydrolytically stable isoelectric membrane having a pI in the range of  $11.5 < \text{pI} < 14$  comprising a hydrolytically stable isoelectric hydrogel having a pI in the  $11.5 < \text{pI} < 14$  range of claim 10, supported on a hydrolytically stable, porous, inert or reactive substrate.

**17.** The hydrolytically stable isoelectric membrane of claim 16, wherein the material of the porous substrate is selected from the group consisting of poly(vinyl alcohol), partially or fully hydrolyzed poly(epihalohydrin), partially or fully hydrolyzed poly(epihalohydrin-co-ethylene oxide), poly(vinyl sulfone), and poly(ether-ether ketone).

- 18.** A method of forming a hydrolytically stable isoelectric membrane having a pI value in the range of  $11.5 < \text{pI} < 14$ , comprising the steps of:
- (a) selecting a carbohydrate-based or polyhydroxy compound-based isoelectric material having a pI value in the  $11.5 < \text{pI} < 14$  range,
  - (b) reacting the isoelectric material at a concentration sufficiently high to set the pH of its aqueous solution equal to its pI value, with:
    - (i) a cross-linker substantially free of ionic functional groups that is structurally different from the isoelectric material, thereby forming an isoelectric hydrogel having a pI value in the range of  $11.5 < \text{pI} < 14$  on a hydrolytically stable substrate.

- 19.** The method of forming a hydrolytically stable isoelectric membrane of claim 18, further comprising the step of:
- (c) reacting the isoelectric material at a concentration sufficiently high to set the pH of its aqueous solution equal to its pI value, with:
    - (i) a cross-linker substantially free of ionic functional groups, and
    - (ii) a hydrophilic polymer substantially free of ionic functional groups that is structurally different from the isoelectric material and the cross-linker.

- 20.** A method of forming a hydrolytically stable isoelectric membrane having a pI value in the  $11.5 < \text{pI} < 14$  range, comprising the steps of:
- (a) selecting a carbohydrate-based or polyhydroxy compound-based precursor having a hydroxyl group with a pKa value in the range of  $11.5 < \text{pKa} < 14$  and having an additional reactive group,
  - (b) selecting a derivatizing agent having a substantially permanently cationic functional group and an additional reactive group, wherein the derivatizing agent is structurally different from the precursor,
  - (c) selecting a concentration of the precursor and the derivatizing agent to establish a desired pI value,
  - (d) selecting a cross-linker having two or more reactive groups, wherein the cross-linker being substantially free of ionic functional groups and structurally different from both the precursor and the derivatizing agent,

(e) reacting the precursor, and the derivatizing agent with the cross-linker,

thereby forming an isoelectric hydrogel having a pI value in the range of  $11.5 < \text{pI} < 14$  on a hydrolytically stable substrate.

**21.** The method of forming a hydrolytically stable isoelectric membrane having a pI value in the  $11.5 < \text{pI} < 14$  range of claim 20, further comprising the steps of:

(f) selecting a hydrolytically stable, hydrophilic polymer having at least two reactive groups that is substantially free of ionic functional groups and structurally different from both the precursor, the derivatizing agent and the cross-linker; and

(g) reacting the hydrolytically stable, hydrophilic polymer, with the precursor, the derivatizing agent and the cross-linker,

thereby forming an isoelectric hydrogel having a pI value in the range of  $11.5 < \text{pI} < 14$  on a hydrolytically stable substrate.

**22.** A hydrolytically stable isoelectric membrane having a pI value in the range of  $11.5 < \text{pI} < 14$  produced by the method of claim 18.

**23.** A hydrolytically stable isoelectric membrane having a pI value in the  $11.5 < \text{pI} < 14$  range produced by the method of claim 20.

**24.** Use of a hydrolytically stable isoelectric membrane having a pI value in the range of  $11.5 < \text{pI} < 14$  according to any one of claims 16, 17, 22 or 23 for an isoelectric trapping separation of an ampholytic compound.